

VAGAL REGULATION OF THE FLOW OF
PANCREATIC JUICE IN THE CAT

by
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Lund 1973

From the Institute of Physiology, University of Lund, Sweden

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To Lester R. Dragstedt



This thesis is based on the following papers which in the text will be referred to by their Roman numerals:

- I. Lenninger, S. & Ohlin, P. (1971). The flow of juice from the pancreatic gland of the cat in response to vagal stimulation. J. Physiol. 216, 303-318.
- II. Lenninger, S. (1971). Effects of parasympathomimetic agents and vagal stimulation on the flow in the pancreatic duct of the cat. Acta physiol. scand. 82, 345-353.
- III. Garrett, J.R., Lenninger, S. & Ohlin, P. (1970). Concerning possible contractile mechanisms in the pancreas - myoepithelial cells. Experientia 26, 741.
- IV. Lenninger, S. (1972). The motility of the pancreatic duct of the cat; an in-vitro study. Acta physiol. scand. 84, 134-141.
- V. Ekström, J. & Lenninger, S. (1973). Choline acetyltransferase and cholinesterase in the pancreatic duct of the cat. Acta physiol. scand. 87, 78-83.
- VI. Lenninger, S. & Olin, T. (1972). The effect of methacholine on the pancreatic duct of the cat studied with a radiological method. Scand. J. Gastroent. 7, 727-731.
- VII. Lenninger, S. (1973). Effects of acetylcholine and papaverine on the secretion and blood flow from the pancreas of the cat. Acta physiol. scand. (in the Press).



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CONTENTS

INTRODUCTION	1
Background and purpose of the present study	3
METHODS	5
General procedures	5
Electrical excitation of the vagus nerve	5
Cannulation and perfusion of the duct	5
Recording of the flow of pancreatic juice and perfusion fluid	6
Measurement of the pancreatic blood flow	6
Determination of proteins in the pancreatic juice	6
Determination of the content of bicarbonate in the pancreatic juice	7
Recording of the motility of the duct in- vitro	7
Estimation of choline acetyltransferase and cholinesterase activities in the duct	8
Measurement of the intraductal pressure	8
Measurement of the width of the duct	8
CHAPTER I	
THE SECRETORY EFFECT OF VAGAL EXCITATION	10
The flow of juice in response to vagal excitation	10
Composition of the juice	11
The transmission of the secretory impulses to the effector cells	12
Parasympathomimetics	12
Eserine	13
Parasympatholytics	14
CHAPTER II	
THE SITE OF ACTION OF THE SECRETORY VAGAL IMPULSES	16
The combined secretory action of cholinergic and	

hormonal stimuli	17
The secretory effect of vagally released gastro-intestinal hormones	19
The effect of vasodilatation on the secretory response to cholinergic stimulation	20
CHAPTER III	
THE DUCTAL EFFECTS OF VAGAL IMPULSES	22
Cholinergic retardation of the flow through the pancreatic duct	22
Retardation caused by movements of the stomach and intestines	23
Retardation caused by increase of proteins in the duct	23
The contractility of the pancreatic duct	24
Motility of the duct	24
Cholinergic innervation of the duct	26
Constriction of the duct in response to cholinergic stimulation	27
SUMMARY AND CONCLUSIONS	30
REFERENCES	33

INTRODUCTION

The function of the exocrine pancreas is regulated by hormones, released from the gastro-intestinal tract and by nervous impulses conveyed to the gland by the vagus, the splanchnic nerves and possibly by local intestino-pancreatic connections. The influence of the extrinsic nerves on the pancreas was first demonstrated and described in dogs by Pavlov and his school (see Babkin, 1928). The importance of the hormones as regulators of the pancreatic secretion became evident with the discoveries of secretin (Bayliss & Starling, 1902) and pancreaticozym (Harper & Raper, 1943). Numerous experiments have since then been performed to define the role played by the nerves and hormones in the regulation of the pancreatic secretion.

The interest in the neural regulation has mainly been focussed on the role played by the vagus nerve. This is due to the fact that this nerve has been shown to cause pancreatic secretion when activated electrically (Pavlov, 1888) or reflexly (Crittenden & Ivy, 1937; Magee, 1959). The interest in the vagus has also been stimulated by the introduction of vagotomy as a method for treating duodenal ulcer in man (Dragstedt & Owen, 1943). A certain number of patients so treated has been found to develop diarrhoea and it has been hypothesized that the vagal decentralization of the pancreas could be responsible for this complication (Burge, 1960; see Barnes & Cox, 1969). To evaluate these findings, a detailed knowledge of the vagal innervation of the pancreas is necessary.

Morphological studies have revealed that preganglionic fibres from the vagus nerve reach the gland by the blood vessels and synapse with the postganglionic neurons within the parenchyma of the gland (Richins, 1945). Postganglionic cholinergic nerves are

distributed around the acinar cells, islets and excretory ducts (Coupland, 1958; Watari, 1968) while the fibres innervating the vessels are adrenergic (Zelander, Ekholm & Edlund, 1962; Lever, Spriggs & Graham, 1968). The adrenergic nerves seem to be confined to the blood vessels (Alm, Cegrell, Ehinger & Falck, 1967).

The influence of the vagus on the pancreas has been studied principally in two ways: by recording the effect of interrupting the vagal impulses by surgical or pharmacological means and by recording the effects of vagal stimulation and parasympathomimetic drugs.

The studies of the effects of vagotomy may yield information on the physiological role of the vagus nerves in the regulation of the pancreatic secretion. The results of these studies are rather conflicting, however. In the dog, the secretory response to acid and foodstuffs in the duodenum has been found to be decreased after vagotomy or atropine in some studies (see Thomas, 1967; Moreland & Johnson, 1971) while in others, both in dog and man, the vagotomy did not affect the pancreatic function (Routley, Mann, Bollman & Grindlay, 1950; Tucker, Barnett & Goodrich, 1964; Holmquist & Colleen, 1965; Henriksen & Rune, 1969). Also the effect of vagotomy on the response to exogenous secretin varies in different studies; in some series the secretion was found to be reduced (Magee, Fragola & White, 1965; Wormsley, 1972) and in other series the secretory response was found to be unimpaired (see Thomas, 1967; White, Lenninger, Elmslie & Magee, 1966; Henriksen, 1969; Moreland & Johnson, 1971).

Vagal excitation has been found to have various effects on the pancreas. As previously mentioned, it has long been known that the vagus nerves may convey impulses which are secretory to the pancreas, i.e. initiate or increase the secretion of water, electro-

lytes and enzymes from the gland. The stimulating effect on the secretion of enzymes is well known (see Thomas, 1950). The vagal influence on the volume flow, on the other hand, is less well understood and seems to vary between different species. Thus, vagal stimulation causes a brisk secretion in the pig (Hickson, 1970a) while only a scanty secretion is seen in the dog (Pavlov, 1888), rabbit (Baxter, 1931) and rat (Lin & Alphin, 1959). The cat has been reported not to secrete at all during vagal stimulation (Korovitsky, 1923; Sergeyeva, 1938; Brown, Harper & Scratcherd, 1967).

Another effect of vagal excitation, reported to occur in the dog (Pavlov, 1888), cat (Korovitsky, 1923) and rabbit (Baxter, 1931) is a retardation of the flow of juice from the gland. The retardation has been attributed to an inhibitory action of certain vagal fibres upon the secretory cells (Pavlov, 1888; Popielsky, 1896) or to mechanical obstruction of the flow in the excretory ducts (Anrep, 1916; Korovitsky, 1923). The latter hypothesis is the currently accepted (Thomas, 1967; Scratcherd, 1969).

The blood flow through the gland is increased during vagal excitation. This has been shown in the dog (Gayet & Guillaumie, 1930b), cat (Hilton & Jones, 1968) and pig (Hickson, 1970b). Hilton and Jones (1968) have suggested that the vasodilatation is caused by kinins, formed in the gland during the neural stimulation.

Background and purpose of the present study. The various effects of vagal stimulation on the pancreas - the secretory, ductal and vascular - do probably not occur independently of each other but influence and modify each other. It is, for instance, easily conceived that constriction of the pancreatic duct may reduce or even completely annul a flow of juice evoked by stimulation of the secretory cells. A pronounced secretory effect, on the other hand, may perhaps mask an effect which is inhibitory to the flow of

juice. Also the secretory and vascular effects of the nerve stimulation may interact; it is likely that the secretory activity induces vascular changes in the gland but also possible that vascular changes affect the secretion. Finally, an interaction of ductal and vascular effects has been suggested from the finding that an increased pressure in the duct may cause the blood flow of the gland to increase (Bennett & Still, 1933).

The aim of the present experiments was to study some of the separate effects of vagal excitation and parasymphomimetic drugs on the exocrine pancreas and to evaluate the degree of interaction of these effects on the flow of juice from the gland. The following problems have particularly been considered: 1) how do vagal impulses cause a flow of juice from the exocrine pancreas, 2) do vascular events affect the flow of juice, 3) by which mechanism may the flow of juice be retarded by vagal impulses and 4) to what extent is the secretory response to vagal excitation influenced by retarding factors?

The studies have been performed on anaesthetized cats and on in-vitro preparations from the pancreatic duct of cats. There is no resting secretion from the pancreas of the anaesthetized cat and this species is therefore suitable for secretory studies. Furthermore, in the cat an inhibiting effect of vagal impulses has clearly been demonstrated (Korovitsky, 1923) and its influence on the secretion should therefore be possible to evaluate. In fact, the generally held view (Thomas, 1967) that vagal stimulation does not cause any flow of juice in this species might be an example of an interaction of two opposing effects of the nerve excitation, a secretory and an inhibitory. A primary task was therefore to investigate if, after all, the excitation of the vagus could cause the pancreas of the cat to secrete.

METHODS

General procedures. The cats were deprived of food since the night before the experiments but had free access to water. Anaesthesia was induced with ether and maintained with chloralose, injected i. v. in a dose of 80 mg/kg. A cannula was inserted in the trachea and the stomach was drained by a tube introduced through the oesophagus. The abdomen was opened by a midline incision and a ligature was tied around the pylorus.

Electrical excitation of the vagus nerve (I, II, III). The vagal trunks were exposed by a left-sided thoracotomy. Both trunks were divided at the lower end of the oesophagus and the posterior (right) trunk was stimulated with square wave shocks of supramaximal strength, a duration of 1.5 msec and varying frequencies.

Cannulation and perfusion of the duct. The main pancreatic duct was cannulated at the site where it enters the duodenal wall. In most experiments the duct was also cannulated in the tail of the gland or, in a few experiments, in the duodenal process of the gland. These latter cannulation sites served as inlets for fluids into the duct system. The placing of two cannulae in the pancreatic duct was made for two reasons: to obtain good control of the drainage of the gland and to allow studies of the flow through the duct.

The duct was perfused either intermittently or continuously. The intermittent perfusions were made with small amounts of saline - about 0.1 ml at a time - from a syringe in order to rinse the duct and to control the effectiveness of the draining cannula. The continuous perfusions were made with a salt solution flowing into the inlet cannula from a reservoir.

Recording of the flow of pancreatic juice and perfusion fluid. The flow of secretion and perfusion fluid were measured dropwise by a photo cell and the rates of flow were continuously recorded on a smoked drum by an ordinate writer (Clementz & Ryberg, 1949). The drops from the cannula of the pancreatic duct had a volume of approximately 0.02 ml. The volume of the drops of pancreatic juice varied in relation to its protein content so that an increase in protein concentration from 1 to 100 mg/ml caused the volume of the drops to decrease with 15 percent. Such a wide range in protein concentration was seldom encountered, however, and the variation in drop size was therefore disregarded in the calculations. All statements of flow rates are based on counted, individual drops.

Measurement of the pancreatic blood flow (VII). A direct method was used in which the drops of blood from the cannulated splenic vein were counted. The size of the drops were about 0.04 ml and the rate was recorded as described above. The blood re-entered continuously into the animals by the right jugular vein. The vessels between the stomach and omentum and the splenic vein were ligated and the spleen was removed. The animals were heparinized. The method measures only a fraction of the total blood flow of the gland but this is adequate for evaluating changes in the flow. It can be roughly estimated that the blood emerges from about half of the glandular mass with an approximate weight of 2 g. The average blood flow can then be calculated to be 50 ml/100g/min in the present experiments. This value is within the range of those found with other methods (see Delaney & Grim, 1966; Eliassen, Folkow & Hilton, 1972).

Determination of proteins in the pancreatic juice. The content of the proteins in the juice was determined primarily in order to characterize the fluid in the pancreatic duct with respect to viscosity and to serve as an indication of adequate cholinergic sti-

mulation. The protein content was determined by measuring the optical density of the diluted juice in a Zeiss spectrophotometer and comparing it with an albumin standard. Due to the dead space of the duct and cannula the changes in protein concentration lagged behind the actual changes in the juice produced by the secretory cells. The spectrophotometric readings should also be interpreted with some caution since materials other than pancreatic proteins can have gained access to the juice through the cannulation sites. It was, thus, observed in some experiments that the juice was contaminated with blood and therefore gave falsely high values of light absorption. In experiments in which eserine was injected into the duct (I) it was observed that some of the samples had a reddish discoloration, possibly due to rubreserine (see Riker, 1954). These samples had to be discarded.

Determination of the content of bicarbonate in the pancreatic juice. The concentration of bicarbonate was determined by titrating the juice with 0.1 n NaOH after it had been boiled in excess of acid. The collection of juice for bicarbonate determination was done under paraffin oil and therefore prevented recording of the drop rate.

Recording of the motility of the duct in-vitro (IV, V). Segments of the pancreatic duct of cats were used for these studies. The ducts were dissected from the glands after the animals had been killed and then either studied directly or stored in refrigerator in Krebs' solution until the following day. The ducts were divided into segments, about 7 mm long, and a number of these segments from various parts of the ducts were then studied. They were placed two at a time in oxygenated Krebs' solution at 37° and connected to force-displacement transducers operating an ink-writing polygraph. Both longitudinal and circular motility was looked for. To study circular contractions the segments were cut in spirals before

they were placed in the bath.

Estimation of choline acetyltransferase and cholinesterase activities in the duct (V). The presence of acetylcholine synthesizing and inactivating enzymes was demonstrated, thus supporting the view that the duct is innervated by cholinergic nerves. The activity of choline acetyltransferase was determined in three pools of pancreatic ducts of 23 cats and in the pancreatic parenchyma of 5 cats. The activity of the enzyme was determined by the method of Hebb (see Nordenfelt, 1963), where choline and continuously formed acetyl-coenzyme A are used as substrate for the enzyme. The acetylcholine formed was assayed on the frog rectus muscle (Feldberg, 1950).

The presence of cholinesterases in the duct was demonstrated by the potentiating effect of eserine on the tension developed by ductal segments. Fresh longitudinal segments from 7 cats were used and they were arranged in a bath as previously described. The effect of eserine was investigated both on the spontaneously developed tension and on the response to exogenous acetylcholine.

Measurement of the intraductal pressure (III). The pressure in the pancreatic duct was recorded with a method used by Emmelin, Garrett and Ohlin (1968) in the study of salivary ducts. The duct was cannulated at the head of the gland and connected via polyethylene tubings with a pressure transducer, operating a polygraph. The pressure in the duct could be set at desired levels, usually 10 - 15 mm Hg, by connecting the cannula to a pressure bottle via a three-way stopcock. The system also permitted recording of the secretion.

Measurement of the width of the duct (VI). A radiological method was developed which permitted measurement of the width of the duct in vivo. Radiograms were taken of the gland while contrast media

were perfused through the duct from the tail to the head of the gland. The perfusion pressure was set so that the side branches of the main duct became barely visible. The exposures were made on industrial film, automatically changed and exposed every 4th sec during a period of one min. A study of the effect of methacholine was performed on 7 cats by taking one series of films before and one after the i.v. injection of the drug. The width of the ducts was measured with a magnifying ruler at three arbitrarily selected points along the duct and the values before and after the injection were compared.

CHAPTER I

THE SECRETORY EFFECT OF VAGAL EXCITATION

The flow of juice in response to vagal excitation

Experiments were performed to investigate if a flow of juice could be obtained from the resting pancreas of the cat in response to vagal excitation (I). The cats were provided with two cannulae in the pancreatic duct and saline was intermittently flushed through the duct in the beginning of the experiments. Secretin was not injected in these cats. Electrical excitation of the posterior vagal trunk caused a flow of pancreatic juice in 30 out of 35 cats tested. This effect was, however, usually not obtained immediately after the operative procedures but only after some time had elapsed. Once a secretory response had been obtained, however, it usually reappeared during the following stimulation periods. A latency of 1 to 3 min was then seen between the onset of the nerve excitation and the appearance of secretion at the tip of the cannula. During the excitation the juice usually dropped at a regular rate and as long as the nerve was excited, although in some experiments the rate declined during the stimulation period. The flow always stopped within a few min after the excitation was discontinued.

The maximal rate of secretion varied greatly between the different animals. Thus, in some only 0.06 ml (3 drops) was recorded during a stimulation period of 10 min while in others one ml or more was produced during that time. The mean secretion for the whole group was found to be 0.36 ml/10 min. This is about 20% of the maximal flow obtained in response to exogenous secretin. It corresponds to 9 μ l/g glandular tissue / min, approximating the mean weight of the glands to 4 g. The secretion of the pancreas of the cat in response to vagal excitation thus seems to be relatively larger than that of the dog but considerably smaller than that of the pig

(Hickson, 1970a).

The rate of secretion was dependent on the stimulation frequency. The lowest frequency to evoke secretion was 3 Hz. With increasing frequencies the secretion increased until maximal rate was reached at 15 Hz in most cats. The rate then tended to decline if the frequency was further increased. A similar relation between the stimulation frequency and the rate of flow of pancreatic juice has been found in the pig (Hickson, 1970a).

Vagal excitation has not previously been reported to evoke a flow of juice from the pancreas of the cat. Korovitsky (1923) mentions that only a drop could occasionally be seen in response to the stimulation but remarks that secretion probably occurs but that it is retained in the gland. Similar results were obtained by Sergeyeva (1938) and Brown, Harper and Scratcherd (1967). No definite explanation can be given for the difference in the results between the previous experiments and the present ones. Several factors may have contributed to the present results: repeated nerve excitation periods in spite of the often negative results in the beginning of the experiment, the dropwise recording of the juice allowing a very precise and immediate registration of the flow and, finally, the flushing of the duct made possible by the double cannulation technique. The importance of the last factor was clearly demonstrated in some experiments in which the flow of juice was seen to start during nerve excitation when the duct was flushed with saline.

Composition of the juice. The juice produced during the vagal stimulation had a high concentration of protein. The concentration was highest in the first samples of juice but then tended to level off. It did not vary systematically with the frequency of the stimulation. A similar observation was made in the pig (Hickson, 1970a).

The concentration of bicarbonate in the juice ranged between 64 and 140 meq/l, i.e. higher than in the blood. There was a tendency to an increase in the concentration of bicarbonate as the rate of the secretion increased. Comparable information on the relation between bicarbonate content and secretory rate during vagal stimulation is available from experiments on the pig and sheep. In the pig the two parameters are positively correlated (Hickson, 1970a) while in the sheep no such correlation was found (Magee, 1961).

The transmission of the secretory impulses to the effector cells. The efferent vagal impulses are in general known to be transmitted to the peripheral effector cells by acetylcholine. This seems to hold true also for the impulses which cause the secretion of enzymes from the pancreas as judged by the effects of parasympathomimetic and parasympatholytic drugs on this secretion (see Thomas, 1950). Some doubt may be cast upon acetylcholine as the transmitter responsible for the watery secretion, however. Thus, the flow of juice in response to parasympathomimetics is sparse and irregular in the dog (see Thomas, 1950; Hickson, 1970a) and reported to be nil in the cat (Korovitsky, 1923; Eisler & Ågren, 1936; Holton & Jones, 1960). Furthermore, the secretory effect of vagal stimulation in the pig is not reduced by atropine even in relatively big doses (Hickson, 1970a). It was therefore of interest to investigate the role played by acetylcholine in the secretory response to vagal excitation in the cat.

Parasympathomimetics. The previous reports that parasympathomimetic drugs do not cause any flow of juice from the pancreas of the cat seemed to agree with the findings that also vagal stimulation was without effect in this respect. In view of the present finding that vagal excitation indeed can cause the gland to secrete, the effects of parasympathomimetic drugs seemed worth while to reinvestigate. Acetylcholine was therefore given close-arterially to 8 cats (VII).

The doses of acetylcholine were 100 μ g, given as continuous injections during periods of about 10 minutes into the coeliac artery. The acetylcholine caused a flow of juice from all the animals in response to at least one out of 3 injections made. The flow was small, however, ranging between 1 and 6 drops during the injection time. Some of the injections did not cause any flow of juice at all and the flushings of the duct seemed to have very little influence. The blood pressure was only little affected by these injections.

The effect of i.v. injected methacholine on the resting pancreas was recorded in a few other experiments (III, VI). The injections were occasionally seen to cause a small flow of juice. The appearance of juice in the duct, as visualized by the radiological method (VI) suggests that this small flow is caused by newly produced juice and is not due to expression of juice from the main duct.

Eserine. Eserine is known to potentiate the effect of acetylcholine by inhibiting cholinesterases. If the acetylcholine is the transmitter causing the watery secretion in response to vagal stimulation one would expect this secretion to be enhanced by the drug. The secretory effect of vagal stimulation at submaximal frequency was therefore investigated in 6 cats before and after the administration of eserine (I). The drug, dissolved in saline in a concentration of 1 mg eserine sulphate/ ml, was injected intraductally according to a modification of the method of Emmelin, Muren and Strömblad (1954). The purpose of administering in this way is to avoid general effects of the drug. It was found that the submaximal vagal stimulation caused about the same amount of juice after as before eserinizaton. The proteins, on the other hand, seemed to increase, indicating that the eserine in fact had reached the parenchyma of the gland. Thus, these experiments did not show that

acetylcholine is involved in the formation of a watery secretion in the cat's pancreas.

Parasympatholytics. Two parasympatholytic drugs, atropine and piperidino-ethyl-diphenyl-azetamide (Hoechst 9980), were given to 11 cats on which the secretory effect of vagal stimulation was studied (I). Hoechst 9980 was chosen because it has been shown to have a highly specific parasympatholytic action without affecting ganglionic transmission even in relatively high doses (Emmelin & Strömbäck, 1957). Both drugs, in a dose of 0.1 mg/kg, reduced significantly the concentration of the proteins of the juice. The rate of secretion was as a rule not affected by this dose, however. After bigger doses the rate tended to decline but significant reduction was not seen until 2 mg/kg of atropine or 5 mg/kg of Hoechst 9980 had been given. The secretion was not completely annulled by these doses, however.

The doses of the parasympatholytic agents which were required to reduce the rate of flow were relatively high compared to those which reduced the output of proteins; they are higher than the dose required to block vagally evoked pancreatic secretion of the dog (see Thomas, 1950). In the pig, on the other hand, atropine in doses up to 3.0 mg/kg has been shown not to reduce the rate of secretion significantly (Hickson, 1970a) whereas the secretion of enzymes was reduced.

The finding that the vagally evoked flow of juice from the cat's pancreas is not reduced until relatively big doses of parasympatholytic agents have been given may suggest that the effect is transmitted by acetylcholine but that the receptors are relatively inaccessible to the blocking agents, according to the "proximity theory" of Dale and Gaddum (1930). It might of course be speculated that some other transmitters are involved as is assumed to be the

case in other "atropine resistant" effects of the vagus in the gastrointestinal tract. The monoamines and purine compounds may be such transmitters. Adrenergic or purinergic neurons have not been described in the pancreatic ganglia or around the secretory cells, however (Alm, Cegrell, Ehinger & Falck, 1967; Burnstock, 1972) and these compounds, with the exception of dopamine in the dog (Hashimoto, Satoh & Takeuchi, 1971) have not been described to evoke any secretory response from the pancreas when injected into the blood stream.

CHAPTER II

THE SITE OF ACTION OF THE SECRETORY VAGAL IMPULSES

In the previous chapter the secretion of the exocrine pancreas of the cat in response to vagal excitation was described and the mechanism of the transmission of the neural impulses to the effector cells was discussed. The question of which these effector cells are will now be considered.

It should be pointed out that the flow of juice in response to vagal excitation in the present experiments is due to the formation of juice by the secretory cells and is not the result of compression of the duct as discussed by Babkin (1924) or of contraction of hypothetical myoepithelial cells. This conclusion is based on the findings that the secretion usually goes on unabated as long as the nerve is excited (I) and that no compensatory retardation of a secretin-induced secretion is seen at the interruption of the excitation (II). In fact, very little compressing capacity was found in the ducts (III, VI) and no myoepithelial cells were found (III).

The most direct way by which the vagal impulses may cause secretion is that the transmitter acts on the secretory cells proper and activates them to expell their secretory products. Hokin and Hokin (1953) suggest that cholinergic drugs cause the secretion of enzymes from the pancreas in this way. The transmitter of the neural impulses may also act in combination with one or several of the gastro-intestinal hormones such as has been shown for the secretion of hydrochlorid acid from the stomach (Olbe, 1964). Such combined actions of two different stimuli may take place at different levels. It may, for instance, be hypothesized that they act on separate receptors but on the same cells in the same way as the gastro-intestinal hormones are proposed to augment each others'

effects (Grossman, 1970). It is also possible that the neural impulses and the hormones act on different target cells and that one action is the prerequisite of the other. An example of such a chain of events is the vagal release of gastrin which causes the oxyntic cells of the fundus to secrete. Another possibility for a combined action of the neural and hormonal stimuli is that the former causes vasodilatation which facilitates the secretory action of the latter. This has in fact been proposed to be the way by which the vagal impulses increase the flow of juice from the pancreatic gland in cats (Brown, Harper & Scratcherd, 1967).

The pattern of the flow of juice in response to vagal excitation as recorded by the ordinate writer in the present studies (I) is suggestive of a direct neural action on the secretory cells. Thus, the secretory activity was closely related to the periods of nerve excitation and stopped soon after the excitation was interrupted. A more gradual cessation of the flow would be expected if hormones were involved. There are, on the other hand, findings suggesting that secretin may in fact be involved. One such finding is the high concentration of bicarbonate in the juice, usually regarded as the hallmark of the effect of secretin on the gland. Another is the finding that the secretion of water and proteins can be dissociated by low doses of parasympatholytics, indicating that two different mechanisms are operating to activate the secretory cells during the vagal stimulation. Experiments were therefore carried out to elucidate the role played by the gastro-intestinal hormones in the pancreatic response to vagal stimulation.

The combined secretory action of cholinergic and hormonal stimuli

The amount of juice secreted by the pancreas during a combined neural and hormonal stimulation has been found to be larger than the sum of the amounts produced by these stimuli when applied separately. In dogs, thus, vagal stimulation increases considerably

the rate of flow of juice produced in response to secretin (Gayet & Guillaumie, 1930a) and secretin potentiates the response to vagal stimulation in dogs and pigs (Thomas, 1950; Hickson, 1970a). In the cat it has been shown that vagal stimulation increases the response to secretin (Brown, Harper & Scratcherd, 1967). Since, however, no secretory effect of cholinergic stimulation alone has been observed previously in this species, the effect of secretin on the response to cholinergic stimuli has not been determined.

The injection of 100 μ g acetylcholine into the coeliac artery was found to cause the pancreas to produce 1-6 drops of juice over a period of ten minutes (VII). When the same dose of acetylcholine was given to the cats during continuous i.v. injection of secretin, the secretion increased by an average of 22 drops. The response was thus augmented several hundred percent when the acetylcholine acted in concert with secretin.

The potentiating effect of secretin on the secretory response to i.v. injected methacholine is apparent from another series of experiments. A very small flow of juice, estimated to less than one drop was seen in response to the i.v. injection of 1 μ g/kg of methacholine (III, VI). Injected to cats which were secreting in response to secretin this dose of methacholine increased the secretory response with 1 to 10 (mean 4) drops (II). The potentiating effect of different doses of secretin was tried in these experiments. It was found that the secretory effect of methacholine was larger when small amounts than when large amounts of secretin were given. A similar finding has been made in the dog (Lin & Ivy, 1957). The finding may suggest that these two types of stimuli may act through a common pathway where the secretion of water is concerned. The results also point to the great influence that secretin has on the secretory effect of cholinergic stimuli.

The secretory effect of vagally released gastro-intestinal hormones

Vagal excitation is known to release gastrin from the antral mucosa (Uvnäs, 1942; see Gregory, 1964) and gastrin has been shown to cause the pancreas to secrete both enzymes and water (Gregory & Tracy, 1964). In the dog it has in fact been proposed that the pancreatic secretory response to sham feeding is entirely due to vagal release of gastrin (Preshaw, Cooke & Grossman, 1966). There is no clear evidence that also secretin and pancreozymin are released in the same way but results have been presented which point in that direction (Schapiro & Woodward, 1965). Findings have also been made which suggest that gastrin may act to release secretin from the duodenum in man (Chisholm, Young & Lazarus, 1969). The possibility that one or more of the hormones are released and take part in the pancreatic response to vagal stimulation in the cat must therefore be considered.

The right vagal trunk was excited in three cats (called "donor cats") so that a flow of pancreatic juice was obtained (I). Through a cannula placed in the portal vein blood was intermittently drawn and injected into the inferior caval vein of three other cats (called "receiver cats"), likewise provided with a pancreatic cannula. Corresponding amounts of blood were at the same time drawn from the inferior caval vein of the receiver cats and then injected into the portal vein of the donor cats. During this exchange of blood there was no secretory response from the receiver cats. The receiver cats started to secrete, however, when they were given equal or even smaller amounts of blood from the donor cats when these were made to secrete in response to intraduodenal installation of hydrochloric acid or i.v. injection of secretin. These stimuli were adjusted so as to cause the pancreas of the donor cats to secrete at a rate similar to that seen during the vagal stimulation. Details of these experiments are given in Fig. 3, paper I.

The transfusion experiments show that vagal stimulation does not release enough secretin or other hormones from the gastro-intestinal tract to cause the pancreas to secrete. The experiments do not preclude, however, that small amounts of hormones are released and that these, in combination with the vagal influence on the gland, may contribute to the activation of the secretory cells. To exclude also this possibility, the vagus should have been excited in animals, in which the antrum and small intestines were removed. This has been done in experiments on the pig (Hickson, 1970a) with maintained secretory response to the stimulation as a result.

The effect of vasodilatation on the secretory response to cholinergic stimulation

Vagal excitation has been shown to cause vasodilatation in the pancreas of the cat (Hilton & Jones, 1968; Barlow, Greenwell, Harper & Scratcherd, 1968). It has been proposed (Brown, Harper & Scratcherd, 1967) that the vagally evoked vasodilatation is the cause of the increased secretion from the secretin-stimulated gland. In order to test this hypothesis the effects of papaverine and acetylcholine on secretion and blood flow were investigated (VII). First the effect of papaverine on the resting gland was studied. The drug was injected continuously into the coeliac artery in doses of 10 mg in periods of 10 min. This did not cause secretion from the pancreas if secretin had not been given. When secretin was then continuously infused, however, the rate of secretion regularly increased during the i.a. injection of papaverine. In experiments in which both the secretion and the blood flow were measured it was found that the secretion, elicited by a moderate dose of secretin and the blood flow increased when papaverine was given. Acetylcholine given into the coeliac artery had a similar effect. Other vasodilating drugs, such as sodium nitrite and histamine have previously been found to increase a secretory response evoked by exogenous secretin (Barlow, 1927; Brown, Harper

& Scratcherd, 1967). These drugs, like papaverine, are not secretory to the pancreas by themselves. Hence, it seems, as if vasodilatation alone cannot cause the gland to secrete and can therefore not be the only way by which vagal impulses and acetylcholine cause the gland to secrete. In the presence of exogenously administered secretin, on the other hand, the vasodilatation caused by the cholinergic stimuli seems to be an important factor to increase the secretory rate.

The way by which vasodilatation in the presence of secretin may increase the rate of secretion is not immediately obvious. It has been proposed that the increase is caused by a facilitated supply of secretin to the gland (Brown, Harper & Scratcherd, 1967). It has also been suggested that the secretion increases owing to an improvement of the nutritional conditions of the gland by the vasodilatation (Barlow, 1927). To evaluate these theories, further studies on the distribution and metabolism of the secretin must be awaited.

CHAPTER III

THE DUCTAL EFFECTS OF VAGAL IMPULSES

Cholinergic retardation of the flow through the pancreatic duct

Vagal excitation and the injection of parasympathomimetic drugs were shown to increase the flow of juice from the secretin-stimulated pancreas of the cat (II, VII). No inhibition was noticed except for an occasional retardation of the first drop after the start of the stimulation. In view of the findings of Korovitsky (1923) and Eisler and Ågren (1936) one might have expected to see a more pronounced inhibition of the flow. It is possible that the secretory effects of the stimulations in the present experiments were so effective that the hindrance to the flow was immediately overcome and therefore not observed. The ductal effects of cholinergic stimulation were further investigated in cats which were not secreting but instead had their pancreatic ducts perfused with a salt solution (II). The solution flowed from a reservoir into the duct of the tail of the gland and was let out through the cannula of the main duct. The pressure was set so that the rate of flow through the duct was similar to that seen during secretion in response to a moderate dose of secretin. Doses of methacholine and frequencies of vagal stimulation were used which had been found to cause secretion in the previous experiments, i.e. 0.1 to 2.0 $\mu\text{g}/\text{kg}$ of methacholine injected i.v. and vagal excitation at a frequency of 8 to 10 Hz. It was found that the methacholine and the vagal stimulation usually caused the rate of flow of the perfusion fluid to decrease markedly. The retardation due to methacholine lasted one to two min, and thereafter the rate of flow usually returned to the pre-injection level. Also the excitation of the vagus for periods of about one min usually retarded the flow as long as the excitation lasted. In some cases no retardation but an increase in the flow rate was seen both after the injection of methacholine and vagal stimulation.

The inhibition of the flow of juice and other fluids has been ascribed to the contraction of smooth muscle in the pancreatic duct (Anrep, 1916; Korovitsky, 1923). This idea was disputed by Gayet and Guillaumie (1933), however, who thought the retardation was due merely to the vagally evoked intestinal movements, displacing or kinking the duct and cannula. A third explanation to be considered, only briefly mentioned in the literature (Bennett & Still, 1933; Hallenbeck, 1967) is that the flow is impeded by an increase in the viscosity of the juice, expected to follow upon the secretion of proteins during cholinergic stimulation. The influence of these factors on the flow was studied.

Retardation caused by movements of the stomach and intestines. It is reasonable to assume that forceful movements of the structures adjacent to the pancreas may affect the flow through the duct, particularly at low rates of flow. It was therefore of interest to see if the flow was retarded also when the stomach and intestines had been removed. This was studied in 5 experiments. In 4 of the animals thus operated on methacholine still caused a retardation of the perfusion. In another series of experiments the influence of the gastro-intestinal movements was avoided by applying the stimulating agents directly in the duct. This was done by injecting acetylcholine and methacholine in doses of between 0.1 and 10 μ g into the perfusion fluid in the inlet cannula. In most of the cases this caused a retardation of the flow lasting between 1 and 3 min.

Retardation caused by the increase of proteins in the duct. Direct evidence is not available to show that increased concentration of proteins in the ductal content may retard the flow but some of the results presented in paper II may suggest it. It was found that the i.v. injection of secretin often caused the rate of flow of perfusion fluid to be markedly retarded. The effluent collected after such an injection had a high content of proteins, reflecting the

well known "washing-out" of enzymes seen after an initial dose of secretin. Because secretin is not known to increase the tension of smooth muscle and, in fact, had no effects on the duct in the in-vitro studies (IV), the retardation of flow seems most readily explained by a changed viscosity of the ductal content. A similar mechanism may contribute to the retardation caused by cholinergic stimuli since they usually caused the protein concentration of the effluent to increase.

The contractility of the pancreatic duct

The resistance to the flow of juice and other fluids through the pancreatic duct was suggested to be influenced by several factors as mentioned in the previous section. Active constriction of the duct is the factor which has attracted most interest (Anrep, 1916; Korovitsky, 1923; Thomas, 1967; Scratcherd, 1969) and is the most likely to be of physiological significance. It has therefore been studied in some detail. Firstly, it was investigated whether the duct actually has the ability to develop active tension and if it is supplied with a cholinergic innervation. Secondly, attempts were made to determine if the pancreatic duct could constrict in response to cholinergic stimulation.

Motility of the duct. Morphological studies have revealed that smooth muscle cells are present in the pancreatic duct of the cat (Eberth, 1863; Richins, 1945; Coupland, 1958; Watari, 1968; Garrett, Alm & Lenninger, 1973). The muscle layer is rather thin and not closely knit and seems to be orientated mainly in a longitudinal, possibly spiral fashion (Eberth, 1863; Garrett, Alm & Lenninger, 1973). It was therefore of interest to see to what extent contractions could be recorded from preparations from the duct and in which direction the contractile forces were operating. These studies were performed on isolated segments of the duct, placed in Krebs' solution and connected to a force-displacement transducer (IV).

Longitudinal, tubular segments of the duct were found to develop active tension and to contract spontaneously. These signs of smooth muscle activity were seen both in fresh preparations and in preparations which had been kept refrigerated since the day before the experiments. Helical strips of the duct, on the other hand, developed no, or only minimal, spontaneous activity. This finding leads to the conclusion, supported by morphological findings (Garrett, Alm & Lenninger, 1973) that the pancreatic duct of the cat has virtually no circular muscle layer. The contractions of some of the tubular segments were irregular in amplitude and frequency. In other segments they were quite regular, however, and the individual contractions then appeared with a frequency of about 2/min and a duration of about 20 sec. They were characterized by a rapid rise in tension and a more gradual relaxation. This type of contraction indicates an integrated activity of the muscle cells in the preparations and does thus suggest that the ductal muscle is a functional syncytium. The spontaneous activity is likely to be myogenic in view of the fact that it could be seen after anoxic, cold storage for more than 24 hr and that it was unaffected by atropine in a concentration which abolished the effect of added acetylcholine.

Acetylcholine stimulated the development of tension in the tubular segments but had almost no effect when tried on the helical strips. The threshold concentration to increase the tension of the tubular segments was usually 10^{-8} g/ml of acetylcholine chloride and maximal response was seen at a concentration of 10^{-5} g/ml. The effects of the acetylcholine in these concentrations were abolished by atropine sulphate (10^{-6} g/ml).

The effects of isoprenaline and noradrenaline were tried in a few experiments. Isoprenaline in concentrations from 10^{-8} g/ml decreased the tension and at higher concentrations the spontaneous

contractions disappeared. These effects were abolished by propranolol, indicating that the cells are provided with β -adrenoceptors. After propranolol a slight increase in the tension was seen when noradrenaline was given, indicating that also α -receptors are present in the duct. These findings do not as such warrant the conclusion that adrenergic nerves take part in the regulation of the motility of the duct. Adrenergic nerves have in fact not been observed in proximity of the smooth muscles of the duct other than in association with the blood vessels of the duct (Garrett, Alm & Lenninger, 1973). On the other hand, it has been found that catecholamines and stimulation of the sympathetic nerves to the pancreas decrease the resistance to the flow through the duct of the cat (Scratcherd, 1969).

Secretin did not affect the tension of the segments. This finding was expected but is nevertheless worth noting with regard to the retardation of the perfusion through the duct seen after the i.v. injection of this hormone (II).

Cholinergic innervation of the duct (VI). The smooth muscle cells of the pancreatic duct of the cat have been shown to be innervated by nerves from the parasympathetic system (Richins, 1945). Recent electrone microscopical and histochemical investigations indicate that these nerves are cholinergic (Watari, 1968; Garrett, Alm & Lenninger, 1973). In order to investigate further the cholinergic innervation of the duct, the activities of choline acetyltransferase and cholinesterases were estimated.

Choline acetyltransferase was found to be present in the ducts in a concentration corresponding to the synthesis of about 100 μ g of acetylcholine/h/g acetone powder. This activity is relatively low compared to that found in other autonomically innervated structures (Nordenfelt, 1965; Ekström, 1970) but shows nevertheless that the

duct is likely to be cholinergically innervated. The activity of the enzyme was also determined in the parenchymatous part of the gland. The enzyme was there found to be present in a concentration corresponding to the synthesis of about 200 μg of acetylcholine/h/g acetone powder, a concentration within the range of that found in the salivary glands of the cat (Nordenfelt, 1963). The finding supports the concept of a cholinergic innervation of the glandular tissue.

The activity of cholinesterases as revealed by the potentiation of acetylcholine by eserine was investigated on the in-vitro preparation of the duct. Eserine sulphate in a concentration of 10^{-6} g/ml caused the spontaneously developed tension to increase in 4 out of 7 ducts investigated. The increase, when it occurred, was small and the results indicate that only small amounts of acetylcholine were released at the time of investigation.

The contractions of the ductal segments in response to exogenous acetylcholine were also measured before and after eserine had been added to the bath. It was found that the ED^{50} of acetylcholine, defined as the dose causing 50 percent of the maximal tension, was lowered by almost a factor of ten after eseriniziation of the segments. The finding is compatible with the idea of a cholinergic innervation of the duct and agrees with the finding that acetylcholinesterase stainable material is present in the nerves of the duct (Garrett, Alm & Lenninger, 1973).

Constriction of the duct in response to cholinergic stimulation.

Evidence has been presented that the pancreatic duct of the cat may exert active movements and that these may be cholinergically regulated. These findings do not a priori permit the conclusion, however, that the activation of the smooth muscles causes a narrowing of the lumen and a retardation of the flow through the duct. Two

series of experiments were therefore performed in which it was tried to determine whether a constriction of the duct occurs in response to cholinergic stimulation.

The effect of cholinergic stimulation on the pressure in the pancreatic duct was studied in 3 cats (III). It was reasoned that a ductal constriction, or possibly a contraction of hypothetical myoepithelial cells in the gland, would affect the pressure in the duct. Vagal stimulation at a frequency of 10 Hz and the i.v. injection of methacholine were found to cause only a slow and gradual increase in the pressure. This effect could not be differentiated from that caused by secretion. A similar increase in pressure was also seen after the injection of a small dose of secretin. No pressure effects, typical of forceful ductal constrictions, were seen.

The pancreatic duct was also studied by a radiological method (VI). The width of the contrast-filled pancreatic ducts of 7 cats was determined from radiograms taken before and after the i.v. injections of methacholine in a dose of 1 μ g/kg. The width of the duct became significantly reduced in 4 of the animals after the injection. In two others the duct became dilated and in one no change was seen. Both the reduction and the widening of the lumen were seen along the whole extension of the duct. There was no sign of segmentation and the lumen was never seen to be completely occluded by constriction.

The results of these two studies suggest that cholinergic stimulation may alter the width of the pancreatic duct of the cat but that the changes are variable. The way by which the longitudinal muscle fibres may constrict the duct is perhaps by a twisting movement, made possible by a spiral arrangement of the muscle cells as proposed by Garrett, Alm and Lenninger (1973). A contraction of

strictly longitudinal fibres, on the other hand, can be speculated to cause a dilatation of the duct as seen in two of the animals. The constriction of the lumen is small, as inferred from the radiograms, but may, nevertheless, be large enough to cause the resistance in the duct to be altered and thereby affect the flow through the duct. It was suggested by Babkin (1924) that the secretory response of the dog's pancreas to vagal stimulation might be due to contraction of the ducts, thus squeezing out its content. In view of the small volume of the pancreatic duct of the cat (VI) and the small reduction of its lumen during cholinergic stimulation, this mechanism will probably not contribute much to the flow of juice from the pancreas of the cat, however.

The significance of the finding that the pancreatic duct can contract is not clear. It can not be taken for granted from the experimental findings that the ductal muscles are activated by the same excitation which normally may influence the secretion of the gland. It is tempting to speculate, however, that the presence of smooth muscles with a motor innervation may have a physiological function. The longitudinally directed contractions are suggestive of a supporting function, perhaps adapted to keep the duct open. This might be appropriate in a gland as flaccid as the pancreas, particularly when the gland is situated intraperitoneally as in the cat. A peristaltic function of the ductal movements is possible although there is, so far, no evidence for such an activity. It may, finally, be hypothesized from the likely assumption that the contractions will occlude the side branches of the main duct that the muscles may exert a protective function by sealing off the parenchyma from noxious factors in the duct.

SUMMARY AND CONCLUSIONS

The experiments have shown that vagal stimulation affects the flow of juice from the pancreas of the anaesthetized cat. The nervous impulses initiate or increase the flow by stimulating the production of juice from the secretory cells. The impulses may also retard the flow by causing an increase in the resistance to the flow in the main ducts of the gland.

A secretory response to electrical excitation of the posterior vagal trunk was seen in 30 out of 35 cats tested. Maximal response was usually obtained by a stimulation frequency of 15 Hz. The mean rate of secretion was then 0.36 ml/10 min which is about 20 percent of maximal secretin-induced secretion. The maximal rates of secretion varied considerably between the individual animals. The secretion did usually not appear immediately after the operative procedures were finished but only when the nerve had been excited repeatedly for some time and the duct had been cleared of its content by intermittent flushings with saline. Once a secretory response had been obtained, however, the following excitations caused a flow of juice within a few minutes and the secretion then persisted as long as the nerve was excited. The concentration of proteins in the juice was high but could not be seen to be related to the frequency of stimulation. The content of bicarbonate of the juice was higher than that of blood.

Relatively large doses of parasympatholytic drugs were required to reduce the rate of secretion in response to vagal stimulation while the content of proteins of the juice decreased already after small doses. This dissociation of volume flow and enzyme content may indicate that the vagally evoked secretion emerges from different types of cells.

Eserinization of the gland did not enhance the secretory response

to submaximal vagal stimulation. This finding, together with the relative resistance of the flow to parasympatholytic drugs, may indicate that acetylcholine is not the transmitter responsible for the watery secretion. It was found, on the other hand, that acetylcholine was able to evoke a secretory response, albeit small, from the resting gland. This may speak in favour of this agent as the transmitter involved.

The watery secretion in response to vagal excitation and parasympathomimetic drugs seems to be evoked in two ways: 1) by activating receptors on the secretory cells proper and 2) by causing vasodilatation in the gland. The latter effect leads to a secretory response only if secretin is available to the secretory cells. It was not found that the secretion in response to vagal excitation was caused by the release of gastro-intestinal hormones.

The secreted volume in response to vagal excitation was relatively small. In view of its high content of enzymes, however, this secretion may nevertheless be physiologically important. Furthermore, when secretin is present in the blood, as will normally be the case during digestion, the augmenting effect of cholinergic stimuli on the secretion is considerable. This effect is most pronounced when only small amounts of secretin are present in the blood. It seems therefore, as if the vagal impulses could provide a means of increasing the output of pancreatic juice without the involvement of large amounts of gastro-intestinal hormones.

The vagal impulses will activate cholinergic neurons which innervate the smooth muscles of the main excretory ducts of the pancreas of the cat. Muscular activity was found along the whole extension of the main duct and the findings indicate that the muscle fibres are arranged in a longitudinal direction and act as a functional syncytium. Cholinergic stimulation causes the muscle

fibres to contract and may thereby alter the width of the duct. Usually the stimulation will cause a slight constriction of the duct but not to the extent that any significant amounts of juice are expressed. The constriction will, on the other hand, increase the resistance to the flow through the duct. Normally, i.e. when the gland is stimulated both neurally and hormonally this increase in resistance will probably be met with an increase in secretory pressure and no retardation of the flow from the gland is noticed. Under experimental circumstances, however, when the gland is exclusively cholinergically stimulated a different situation may ensue. The juice produced in response to such stimulation is relatively scarce but rich in enzymes, particularly in the beginning of a stimulation. The relatively high viscosity of this juice and the simultaneous narrowing of the width of the duct may then offer too great a resistance for the secretion to overcome. This may be one of the explanations why vagal stimulation has been reported not to cause any flow of juice from the pancreas of the cat.

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